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Research article

Combined effect of intermittent hemostasis and a modified external hemorrhage control device in a lethal swine model

Hua-yu Zhang [a], Yong Guo [a,b], Dong-chu Zhao [a], Xiao-ying Huang [a], Yang Li [a], Lian-yang Zhang [a,*]

[a] Department of Trauma Surgery, Medical Center of Trauma and War Injury, State Key Laboratory of Trauma, Burns and Combined Injuries, Daping Hospital, Army Medical University (Third Military Medical University), Chongqing, China
[b] Emergency and Critical Care Center, The Third Affiliated Hospital of Chongqing Medical University, Chongqing, China

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ABSTRACT

Background: Non-compressible torso hemorrhage (NCTH) presents the ultimate challenge in pre-hospital care. While external hemorrhage control devices (EHCDs) such as the Abdominal Aortic and Junctional Tourniquet (AAJT) and SAM Junctional Tourniquet (SJT) have been invented, the current design and application strategy requires further improvement. Therefore, researchers devised a novel apparatus named Modified EHCD (M-EHCD) and implemented intermittent hemostasis (IH) as a preventive measure against ischemia-reperfusion injury. The objective of this study was to ascertain the combined effect of M-EHCD and IH on the hemostatic effect of NCTH.
Methods: Eighteen swine were randomized to M-EHCD, AAJT or SJT. The NCTH model was established by inducing Class III hemorrhagic shock and performing a hemi-transection of common femoral artery (CFA). EHCDs were rapidly fastened since the onset of free bleeding (T_{0min}). The IH strategy was implemented by fully releasing M-EHCD at T_{40min}, T_{70min} and T_{100min}, respectively, whereas AAJT and SJT maintained continuous hemostasis (CH) until T_{120min}. All groups underwent CFA bridging at T_{110min}, and EHCDs were removed at T_{120min}. Reperfusion lasted for 60 min, after which euthanasia was performed. Hemodynamics, intra-vesical pressure (IVP), and blood samples were collected periodically. Histological examinations were also conducted.
Results: M-EHCD demonstrated the fastest application time (M-EHCD: 26.38 ± 6.32s vs. SJT: 30.84 ± 5.62s vs. AAJT: 54.28 ± 5.45s, $P < 0.001$) and reduced free blood loss (M-EHCD: 17.77 ± 9.85g vs. SJT: 51.80 ± 33.70g vs. AAJT: 115.20 ± 61.36g, $P = 0.011$) compared to SJT and AAJT. M-EHCD exhibited inhibitory effects on heart rate (M-EHCD: 91.83 ± 31.61bpm vs. AAJT: 129.00 ± 32.32bpm vs. SJT: 135.17 ± 21.24bpm, $P = 0.041$) and shock index. The device's external pressure was lowest in M-EHCD and highest in SJT ($P = 0.001$). The resultant increase in IVP were still the lowest in M-EHCD (M-EHCD: −0.07 ± 0.45 mmHg vs. AAJT: 27.04 ± 5.03

Abbreviations: NCTH, non-compressible torso hemorrhage; EHCD, external hemorrhage control device; AAJT, Abdominal Aortic and Junctional Tourniquet; SJT, SAM Junctional Tourniquet; PE, pressurized element; IRI, ischemia-reperfusion injury; REBOA, Resuscitative Endovascular Balloon Occlusion of the Aorta; M-EHCD, Modified External Hemorrhage Control Device; IH, intermittent hemostasis; CH, continuous hemostasis; CFA, common femoral artery; EIA, external iliac artery; CVP, central venous pressure; IVP, intra-vesical pressure; MAP, mean arterial pressure; DEP, device's external pressure; ANOVA, one-way analysis of variance; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; SI, shock index; Lac, lactate; TEG, thrombelastogram; AC, abdominal compliance.

* Corresponding author.
E-mail addresses: zhanghuayu1024@163.com (H.-y. Zhang), m13882395330@163.com (Y. Guo), cqykdxzhaodongchu@outlook.com (D.-c. Zhao), 276361950@qq.com (X.-y. Huang), liyang360@163.com (Y. Li), dpzhangly@163.com (L.-y. Zhang).

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mmHg vs. SJT: 5.58 ± 2.55 mmHg, $P < 0.001$). Furthermore, M-EHCD caused the least colonic injury (M-EHCD: 1.17 ± 0.41 vs. AAJT: 2.17 ± 0.41 vs. SJT: 2.17 ± 0.41, $P = 0.001$). The removal of M-EHCD showed the slightest impact on pH ($P < 0.001$), while AAJT group was more susceptible to the lethal triad based on the arterial lactate and thrombelastogram results.
Conclusions: M-EHCD + IH protected the organs and reduced the risk of the lethal triad by decreasing disruptions to IVP, hemodynamics, acid-base equilibrium and coagulation. M-EHCD + IH was superior to the hemostatic safety and efficacy of AAJT/SJT + CH.

1. Introduction

Torso trauma is a significant concern in military medicine and emergency care. Extremity tourniquets are effective in reducing mortality caused by limb injuries [1]. The lack of bony protection makes the axial major vessels, parenchymal organs, and vessels located within the junctional areas more vulnerable to high-energy injury mechanisms, such as improvised explosive devices and motor collisions [2]. Non-compressible torso hemorrhage (NCTH) is difficult to control via conventional compression due to the specific anatomical structures. Notably, NCTH poses a significant challenge in pre-hospital care due to its high incidence, mortality rate, and the limited knowledge and skills among medical staff required to manage it effectively [3–5].

External hemorrhage control devices (EHCDs), such as the Abdominal Aortic and Junctional Tourniquet (AAJT) and SAM Junctional Tourniquet (SJT), were developed to externally occlude internal hemorrhage using pressurized elements (PEs) [6]. While training are also needed, EHCDs play a critical role in managing NCTH within pre-hospital settings. Additionally, the introduction of vascular localization strategies has the potential to enhance the safety of prolonged hemostasis, although there has been little change in the design of these devices [7–9].

However, there are some drawbacks that need to be improved. During the process of hemostasis, EHCDs may potentially cause damage to themselves and inflict severe pain upon patients, which may pose critical obstacles to patient compliance and safety [6,10]. The observed organ damage in experimental animals highlights the important of limiting compressive strength and area [8,11], as well as optimizing the prevention of ischemia-reperfusion injury (IRI) to improve the prognosis.

In recent years, Resuscitative Endovascular Balloon Occlusion of the Aorta (REBOA) has become increasingly significant in NCTH-oriented studies due to its efficient hemostasis and hemodynamically-friendly nature [12]. However, the use of field and en-route REBOA is still limited due to the highly skilled catheterization operations and increased fluid requirements [13,14]. Intermittent REBOA [15], also known as typical ischemic post-conditioning [16], can provide survival benefits and tissue protection effects. This hemostatic strategy may also play a protective role in external NCTH control and promote a sequential pattern of transitioning from pre-hospital EHCD to in-hospital REBOA [17].

In conclusion, there are two tasks that need to be addressed to improve external hemostasis: (1) Enhancing the design of EHCDs; and (2) optimizing iatrogenic prevention. This study presents a novel EHCD, Modified EHCD (M-EHCD), as well as the preventative strategy of intermittent hemostasis (IH), to comprehensively improve the safety and efficacy of external NCTH control.

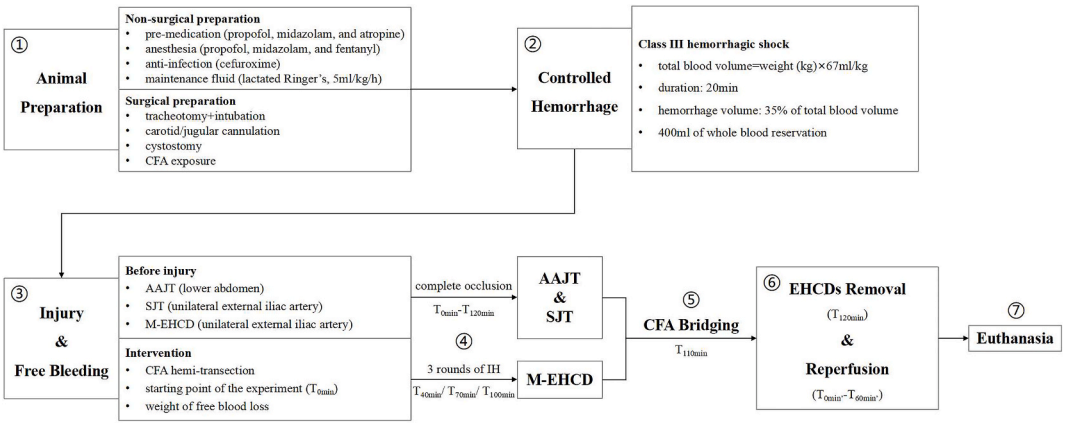

Fig. 1. The flow-chart of this study This study comprised four main stages: animal preparation, controlled hemorrhage, intervention, and reperfusion. T_{0min} was commenced when free bleeding began. CFA bridging was conducted at T_{110min}, followed by EHCDs removal at T_{120min}. $T_{0min'}$ was reset when the reperfusion phase ensued, and the animals were euthanized at $T_{60min'}$.

2. Materials and methods

2.1. Study overview

This study was a prospective randomized trial approved by the Laboratory Animal Welfare and Ethics Committee of the Third Military Medical University (AMUWEC20201513). All subjects were treated humanely in accordance with the principles of *The Guide for the Care and Use of Laboratory Animals* [18].

The experiment consisted of four main stages: animal preparation, controlled hemorrhage, intervention, and reperfusion (Fig. 1 and Supplementary Table 1). The baseline was established at the end of the animal preparation phase. The starting point, T_{0min}, was set at the initiation of free bleeding. The animal size (6 per group) was based on previous literature [19,20]. The animals were randomly assigned to M-EHCD + IH, AAJT + continuous hemostasis (CH), or SJT + CH. The injured side of the common femoral artery (CFA) was determined by second-round randomization after cystostomy.

Two factors impede the advancement of NCTH management. At the hardware level, the existing external hemorrhage control device must be optimized in order to enhance its hemostatic efficacy. At the software level, the IRI induced by CH also has an adverse impact on the rehabilitation process and overall outcomes. It is therefore imperative to refine the device design and hemostatic strategy. This study addresses both of these two aspects concurrently, with the aim of improving the quality of massive bleeding control. In consideration of the aforementioned research intention and the objective of reducing the use of animals [21], M-EHCD + CH and AAJT/SJT + IH were excluded.

2.2. The M-EHCD

The resin pads and T-shaped alloy PE were produced using 3D printing technology. Two triangular pads were positioned on the animals' abdomen and back. Afterwards, the PE was glided through the slot on the ventral pad to reach the optimal location for targeting the bleeding area. The PE's locking bolt was then secured. The researchers took measures to enhance the triangular stability by tightening the nylon belts to prevent any potential dislocation or slackening during hemostasis. The transverse bar of the PE was rotated clockwise until complete blood occlusion was achieved (Fig. 2).

2.3. Animal preparation

Eighteen castrated male Landrace swine weighting between 50 kg and 70 kg were purchased from the Animal Center of Daping Hospital (Chongqing, China) and housed for a minimum of seven days for appropriate acclimation. The animals were fasted overnight and given unrestricted access to water before surgery. Each animal received a pre-medication via one auricular vein, consisting of 1.33 mg/kg propofol, 0.17 mg/kg midazolam, and 0.5 mg atropine. Anesthesia was pumped via the same intravenous line using propofol

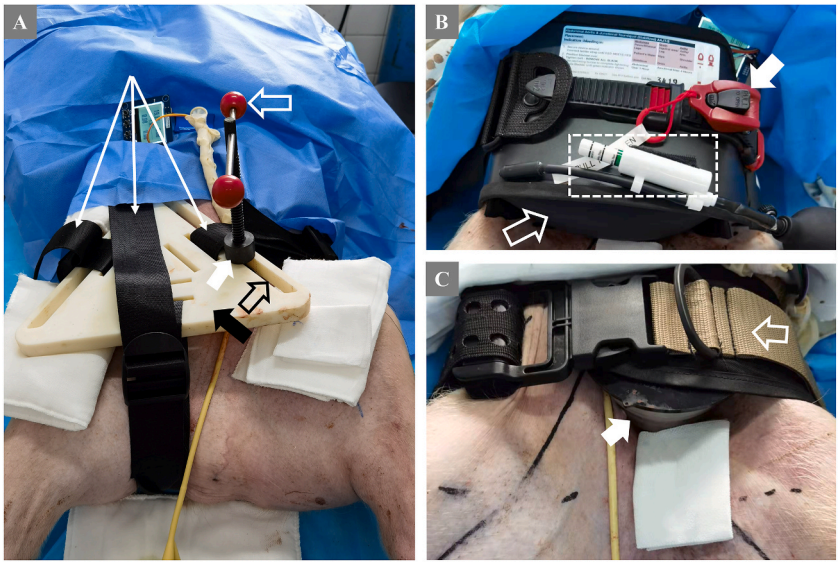

Fig. 2. The EHCDs used in this study A and B: M-EHCD's ventral and dorsal pads envelop the torso, while the belts improve the triangular stabilization. Securing the PE with the locking bolt (dashed box) and turning the horizontal bar (white arrow) clockwise to apply downward pressure. C: The alloy PE of M-EHCD is smaller than the inflatable balloons of AAJT and SJT. D: M-EHCD's PE can block the external iliac artery (EIA) on either side through the chute. E: After firmly fixing AAJT by tightening the belt (white arrow) and the ratchet (dashed box) in turn, the balloon is inflated using an air injector (hollow arrow). AAJT cannot block unilateral EIA due to the balloon's size. F: Fasten SJT's belt (white arrow) until the buckle (dashed box) clicks, then compress the ipsilateral EIA of the injured CFA. Hollow arrow: the air injector.

(4–12 mg/kg/h), midazolam (0.15 mg/kg/h), and fentanyl (4–8 μg/kg/h). Lactated Ringer's solution was administered as maintenance fluid at a rate of 5 ml/kg/h through the contralateral auricular vein after infusion of cefuroxime (1.5g) [22,23]. The mechanical ventilator (Shangrila 590, Aeonmed, Beijing, China) was then activated following midline tracheotomy and intubation. Ventilation frequency, oxygen concentration, peak inspiratory pressure, and positive end-expiratory pressure were set to 15-17bpm, 21 %, 16-18cmH_2O, and 3-4cmH_2O, respectively.

The Seldinger technique was used to intubate the cervical vessels. Body temperature and hemodynamics were monitored through the left common carotid artery, while central venous pressure (CVP) was measured through the left internal jugular vein. The right carotid artery was accessed for arterial sample collection and quantitative exsanguination, and the right jugular vein was cannulated for the transfusion of resuscitation fluid and autologous blood. To maintain the body temperature above 36 °C, a thermal blanket was utilized. For urinary catheterization and intra-vesical pressure (IVP) measurement [24], cystostomy was performed. Lastly, a 4 cm segment of the CFA was surgically isolated.

2.4. Controlled hemorrhage

A modified model of Class III hemorrhagic shock was established to simulate the pathophysiological changes of NCTH [25]. Within 20 min, 35 % of the total blood volume (weight × 67 ml/kg) was manually drawn, and 400 ml of which was stored in citrated bags. However, hemorrhage had to be suspended when the mean arterial pressure (MAP) dropped below 40 mmHg to ensure survival. Blood withdrawal resumed when MAP automatically returned to over 40 mmHg.

2.5. Intervention

EHCDs were placed loosely around the lower torso before CFA injury. Both M-EHCD and SJT were able to occlude bleeding by compressing the ipsilateral external iliac artery, despite their differing designs. However, the AAJT had the potential to affect bilateral flow when inflated. A hemi-transecting of the CFA was conducted using scissors. Subsequently, the induction of free bleeding and the commencement of the experimental timeline (T_{0min}) were initiated. Then the EHCDs were rapidly tightened following the manufacturers' instructions. The researcher documented the interval between T_{0min} and the cessation of active bleeding, as observed through visual assessment, and defined this as the time to hemostasis. The amount of blood loss was determined by measuring the net weight of the gauzes. In order to achieve the objective of a 60-min reperfusion observation period, a segment of infusion tube (length × inner diameter: 4 cm × 3 mm) with both ends trimmed into a wedge shape was inserted into the distal and proximal ends of the injured CFA to restore the vascular continuity at T_{110min}. Subsequently, the ends of the bridging tube were secured with 4-0 sutures external to the CFA wall, thus preventing the potential for secondary bleeding due to slippage. Additionally, the blood loss during the application of EHCDs was also measured.

The continuous blockage of blood flow from T_{0min} to T_{120min} in both AAJT and SJT groups was defined as CH. The implementation of IH differed significantly. M-EHCD was depressurized slowly within 2 min at T_{40min}, T_{70min}, and T_{100min}, respectively. After a 3-min pressure-free period, the original manometer reading (placed between EHCD and body surface) was rapidly restored.

2.6. Reperfusion

The timer was reset to $T_{0min'}$ when EHCDs were gradually released at T_{120min}. Pressurized autotransfusion was initiated at $T_{0min'}$, followed by a bolus infusion of 500 ml of lactated Ringer's solution. Successful arterial bridging was indicated by the palpable pulsation of the injured CFA. Euthanasia was performed humanely at $T_{60min'}$.

2.7. Fluid therapy and outcomes

The planned fluid support included the maintenance fluid since preparation stage, along with 400 ml of autologous transfusion and 500 ml of lactated Ringer's solution during the reperfusion phase. Any additional lactated Ringer's solution administered due to MAP<40 mmHg since the intervention stage was referred to as unplanned fluid resuscitation. It is important to note that no additional fluid was required during IH active period.

The primary outcomes of this study were the combined effects of M-EHCD and IH on the safety and efficacy of NCTH control (Supplementary Fig. 1). Hemodynamic performance, blood samples (Supplementary Table 2), and IVP were collected at baseline, immediately after controlled hemorrhage, and every 30 min since T_{30min} thereafter. The device's external pressure (DEP) was recorded at the end of pressurization and every 30 min from T_{30min} to T_{120min} using a manometer. Urine output was measured at $T_{60min'}$. A single-blinded pathologist analyzed tissue samples harvested from the compressed skin, urinary bladder, penis, small intestine, and colon using hematoxylin eosin stain. Tissue damage was rated on a scale of 0–3 points, with 0 indicating no damage and 1, 2, or 3 indicating mild, moderate, or severe damage, respectively, based on the designated expert's clinical experiences.

2.8. Statistical analysis

SPSS version 25.0 (SPSS, Chicago, USA) was employed to analyze the data. Mean ± standard deviation was used to present continuous variables. For inter- or intra-group comparations, one-way analysis of variance (ANOVA), repeated two-way ANOVA, and Chi-squared test were performed. Significant differences were observed when $P < 0.05$.

3. Results

3.1. Time to hemostasis, blood loss, urine output, and fluid administration

The blood loss over the course of the experiment was divided into four distinct components (Supplementary Table 3): controlled blood loss, free bleeding loss, blood loss during the application of EHCDs (from the achievement of hemostasis to T_{120min}) and the sum of blood loss since T_{0min}. There were no significant differences in controlled blood loss among the three groups (M-EHCD: 1345.00 ± 87.35 ml vs. AAJT: 1301.67 ± 80.10 ml vs. SJT: 1361.67 ± 93.68 ml, $P = 0.486$), as the baseline parameters were consistent. M-EHCD had superior hemostatic time (M-EHCD: 26.38 ± 6.32s vs. SJT: 30.84 ± 5.62s vs. AAJT: 54.28 ± 5.45s, $P < 0.001$) and the lowest free bleeding loss (M-EHCD: 17.77 ± 9.85g vs. SJT: 51.80 ± 33.70g vs. AAJT: 115.20 ± 61.36g, $P = 0.011$) compared to the other two groups. Although the M-EHCD group demonstrated an increase in blood loss since the achievement of free bleeding caused by three rounds of IH (M-EHCD: 85.22 ± 5.31g vs. SJT: 16.92 ± 17.88g vs. AAJT: 15.85 ± 19.59g, $P < 0.001$), no significant inter-group differences in total blood loss since T_{0min} were observed across the three groups (M-EHCD: 102.99 ± 14.48g vs. SJT: 68.72 ± 45.29g vs. AAJT: 131.05 ± 53.34g, $P = 0.059$). Moreover, the effects of EHCDs on both urine output (M-EHCD: 321.67 ± 142.04 ml vs. SJT: 453.33 ± 188.96 ml vs. AAJT: 485.00 ± 108.58 ml, $P = 0.170$) and fluid administration (M-EHCD: 2356.67 ± 271.12 ml vs. SJT: 1683.33 ± 396.92 ml vs. AAJT: 2126.67 ± 840.80 ml, $P = 0.140$) were found to be insignificant.

3.2. Hemodynamic performance

The heart rate (HR) exhibited a significant increase following the establishment of this shock model, which gradually subsided between T_{0min}-T_{120min}. According to the repeated measure, the M-EHCD group had a lower HR compared to the AAJT and SJT groups since T_{30min} ($P = 0.010$). Additionally, the M-EHCD group exhibited the lowest increase in HR among all groups (ΔHR = peak-baseline, M-EHCD: 91.83 ± 31.61bpm vs. AAJT: 129.00 ± 32.32bpm vs. SJT: 135.17 ± 21.24bpm, $P = 0.041$) (Fig. 3A). All EHCDs reversed the trends of deterioration in systolic blood pressure (SBP), diastolic blood pressure (DBP), MAP and CVP caused by hemorrhagic shock (Fig. 3B–E). However, only M-EHCD ($P = 0.132$) maintained the stability of DBP since T_{120min}, whereas reperfusion induced significant DBP fluctuations in AAJT ($P = 0.009$) and SJT ($P = 0.018$) (Fig. 3C). Similarly, the animals' MAP in AAJT group was significantly lower than that of the other EHCDs at $T_{30min'}$ and $T_{60min'}$, respectively (Fig. 3D). Compared to AAJT ($P = 0.020$) and SJT ($P = 0.020$), M-EHCD ($P = 0.715$) also exhibited a favorable impact on the consistency of the shock index (SI, SI=HR ÷ SBP) (Fig. 3F).

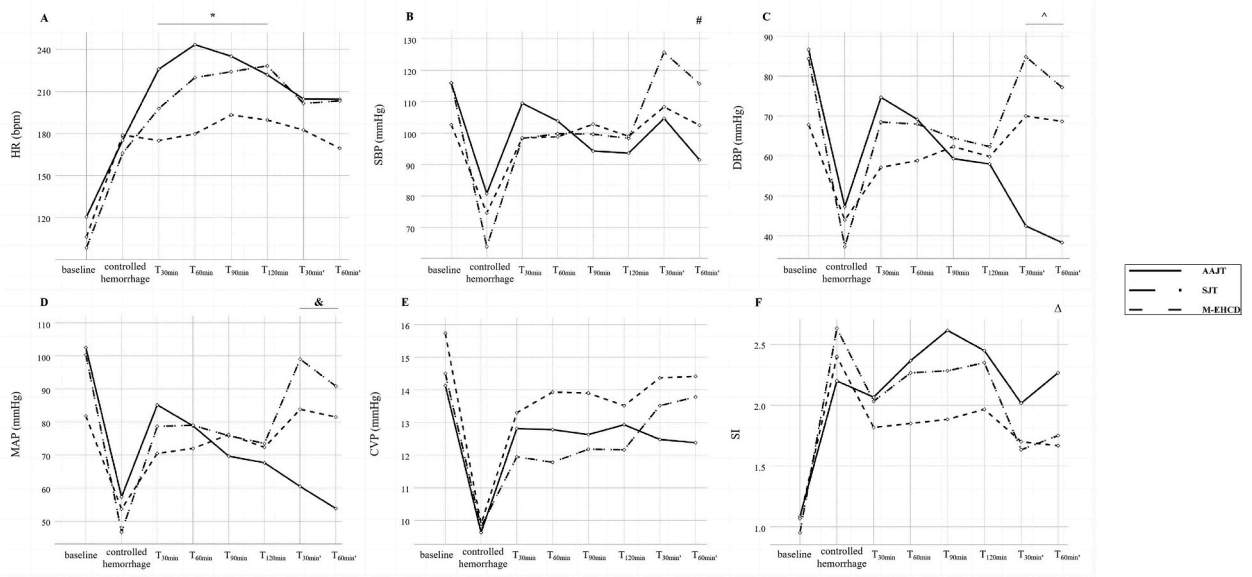

Fig. 3. Hemodynamic performance A: EHCDs were found to alleviate the increase in HR caused by hemorrhagic shock. *: HR of the M-EHCD group was lower than that of the AAJT and SJT groups during hemostasis (T_{30min}-T_{120min}), $P < 0.05$. B: All EHCDs were able to maintain consistency of SBP variation among groups during T_{30min}-T_{120min} ($P = 0.981$). #: SJT had the highest SBP measured at $T_{60min'}$ (SJT: 115.67 ± 12.56 mmHg vs. M-EHCD: 102.50 ± 7.71 mmHg vs. AAJT: 91.50 ± 20.65 mmHg, $P = 0.038$). C: Only animals from the M-EHCD ($P = 0.132$) group were able to maintain the stability of DBP since T_{120min}. ^: In the last two measurements, DBP of the AAJT group was significantly lower than that of SJT and M-EHCD, $P < 0.05$. D: Both the M-EHCD ($P = 0.166$) and AAJT ($P = 0.073$) were able to maintain the intra-group stability of MAP since T_{120min}. &: During the reperfusion stage, MAP of the AAJT group was significantly lower than that of the other two groups, $P < 0.05$. E: The application of EHCDs could reverse the downward trend of CVP. F: M-EHCD had the lowest SI compared to the other groups since $T_{30min'}$ ($P = 0.014$). Δ: At $T_{60min'}$, SI of the M-EHCD group was markedly lower than that of the AAJT and SJT groups (M-EHCD: 1.67 ± 0.31 vs. AAJT: 2.27 ± 0.16 vs. SJT: 1.75 ± 0.34, $P = 0.005$).

3.3. DEP, IVP, and histologic examination

With the exception of AAJT ($P < 0.001$), both M-EHCD ($P = 0.207$) and SJT ($P = 0.249$) demonstrated the capacity to maintain intra-group stabilization of DEP following pressurization to T_{120min}. We assumed that reducing fluctuations in external pressure may provide the necessary support to enhance the stability of external NCTH control. M-EHCD, which required the lowest DEP for hemostasis at ≤35N, was followed by AAJT at 40–45N and SJT at approximately 50N, $P = 0.001$ (Fig. 4A). In addition, EHCD pressurization caused varying degrees of increment in IVP (ΔIVP = T_{30min}-baseline) among groups (M-EHCD: −0.07 ± 0.45 mmHg vs. AAJT: 27.04 ± 5.03 mmHg vs. SJT: 5.58 ± 2.55 mmHg, $P < 0.001$) (Fig. 4B). M-EHCD caused less colonic damage compared to SJT and AAJT based on histopathological assessment (M-EHCD: 1.17 ± 0.41 vs. AAJT: 2.17 ± 0.41 vs. SJT: 2.17 ± 0.41, $P = 0.001$) (Fig. 5). No significant differences were observed in the other tissue injury scores among groups.

3.4. Blood sample tests

The arterial pH remained unaffected by M-EHCD ($P = 0.051$) or SJT ($P = 0.064$) during the experiment. In contrast, the AAJT group displayed the most significant pH variation after reperfusion (ΔpH = $T_{30min'}$-T_{120min}, M-EHCD: −0.05 ± 0.04 vs. −0.27 ± 0.09 vs. SJT: −0.07 ± 0.08, $P < 0.001$) (Fig. 6A). Additionally, only M-EHCD ($P = 0.146$) did not lead to significant changes in lactate concentration (Lac) intra-group observations until $T_{60min'}$. The difference in Lac (ΔLac = $T_{30min'}$-T_{120min}) among the three groups (M-EHCD: 2.59 ± 2.31 mmol/L, AAJT: 8.15 ± 1.87mmol/, and SJT: 1.70 ± 1.73 mmol/L, $P < 0.001$) indicated that AAJT was inadequate in maintaining the homeostasis of Lac, as evidenced in the reperfusion phase (Fig. 6B).

Venous potassium levels increased gradually in all groups from T_{90min} onwards, with the most significant change observed in the AAJT group ($P = 0.010$) (Fig. 6C). During the observation period of T_{30min} to T_{120min}, the M-EHCD group exhibited a consistently mild elevated D-dimer level in comparison to the other groups ($P = 0.005$). Nevertheless, the notable elevation of D-dimer in the AAJT group following the removal of EHCDs ($P = 0.008$) suggested a heightened risk of secondary hyperfibrinolysis in this group during reperfusion (Fig. 6D).

The thrombelastogram (TEG) data collected at the study endpoint indicated an elevated risk of coagulopathy within the AAJT group (Fig. 6E–J). The notable alterations in R value (AAJT: 5.90 ± 1.25min vs. M-EHCD: 4.20 ± 2.64min vs. SJT: 3.25 ± 0.73min, $P = 0.007$), MA value (AAJT: 66.72 ± 6.18 mm vs. M-EHCD: 74.38 ± 2.65 mm vs. SJT: 73.42 ± 3.78 mm, $P = 0.018$), and G value (AAJT: 10.40 ± 2.40Kd/sc vs. M-EHCD: 14.83 ± 2.13Kd/sc vs. SJT: 14.15 ± 2.74Kd/sc, $P = 0.014$) demonstrated that AAJT may have a detrimental impact on the formation or stability of blood clots. The inverse trends of K value ($P = 0.041$) and Angle value ($P = 0.004$) suggested that downstream blood flow recirculation may increase the likelihood of fibrin dysfunction. Moreover, the final CI value (AAJT: −0.42 ± 4.86 vs. M-EHCD: 3.48 ± 2.38 vs. SJT: 4.30 ± 1.09, $P = 0.048$) may also provide a clue of hyperfibrinolysis induced by AAJT.

Other parameters such as hematocrit, TNF-α, aspartate transaminase, alanine transaminase, lactate dehydrogenase, blood urea nitrogen, creatinine, creatine kinase and troponin T were statistically insignificant among groups during the study.

4. Discussion

4.1. Major findings

We developed a Class III hemorrhagic shock model to simulate NCTH based on the literature and diagnostic criteria [2,25]. The current data clearly demonstrated that the experimental animals all exhibited severe hemodynamic changes after controlled

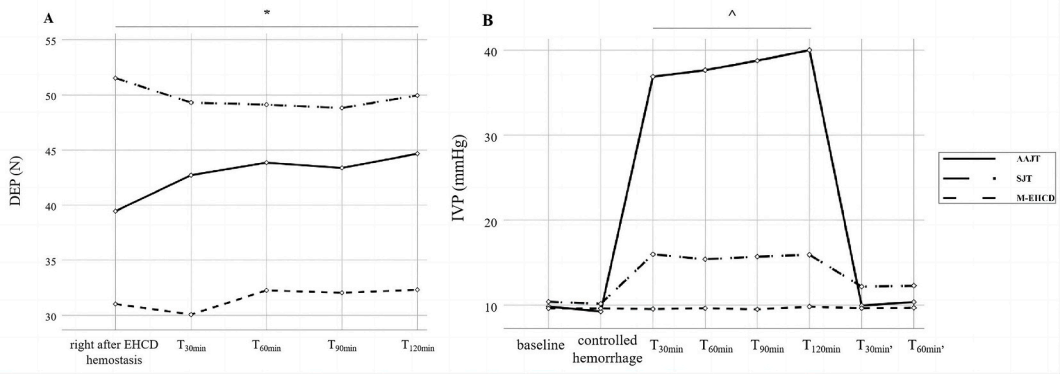

Fig. 4. DEP and IVP measurement A: Both M-EHCD ($P = 0.207$) and SJT ($P = 0.249$) were able to maintain intra-group stability of DEP. *: DEP generated by M-EHCD was lower than that of SJT and AAJT during EHCD hemostasis ($P < 0.005$). B: The M-EHCD group exhibited the lowest increase in IVP among groups when EHCD was applied ($P < 0.001$). ^: The four IVP measurements of the AAJT group were significantly higher than those of SJT and M-EHCD ($P < 0.001$).

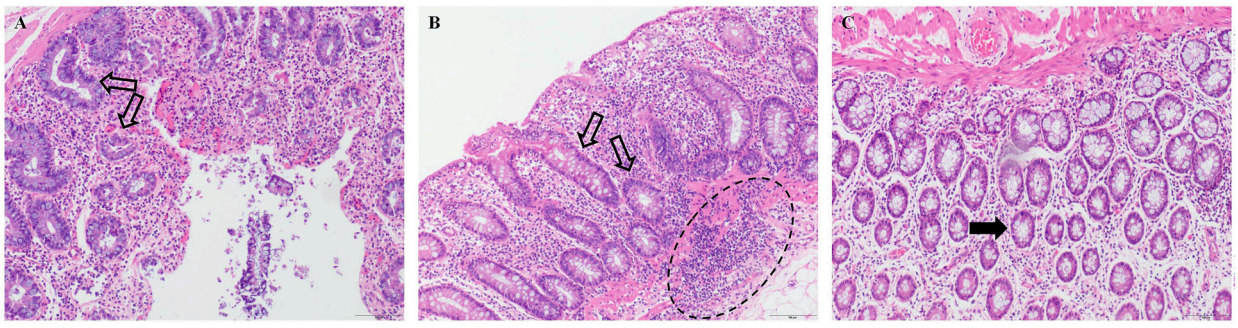

Fig. 5. Histologic examination A–C: Microscopic examinations of the colon collected from the AAJT, SJT, and M-EHCD groups were presented respectively. According to the pathologist's assessment ($P = 0.001$), M-EHCD resulted in the least damage to the colon. A decreased number and poor morphological homogeneity of goblet cells were observed (hollow arrow). Additionally, the infiltration of inflammatory cells within the lamina muscularis mucosae was evident (dashed line). Conversely, a large number of goblet cells with normal morphology were present (solid arrow). Scale bar: 100 μm.

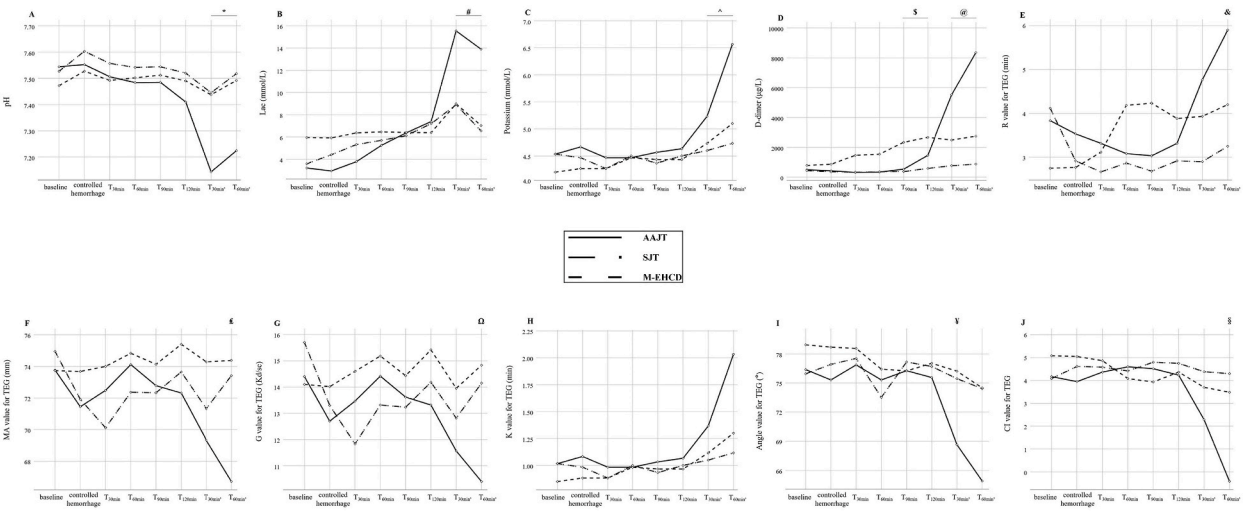

Fig. 6. Blood sample tests A: The pH declined during reperfusion and gradually increased until $T_{60min'}$. *: The pH of animals in the AAJT group was consistently the lowest among groups starting from $T_{30min'}$ ($P < 0.005$). B: The increase in Lac in the AAJT group was more pronounced compared to the other groups when reperfusion started ($P < 0.001$). #: The highest levels of Lac were observed in the AAJT group during the last two measurements ($P < 0.005$). C: The AAJT group showed the most substantial change in potassium levels between T_{90min}-$T_{60min'}$ ($P = 0.010$). ^: After the removal of EHCDs, the AAJT group had higher potassium levels than the SJT and M-EHCD groups ($P < 0.005$). D: The M-EHCD group had higher D-dimer levels than other groups during T_{90min}-T_{120min} ($: $P < 0.05$), @: this trend was reversed by the AAJT group since $T_{30min'}$ ($P < 0.05$). E: The value of R, evaluated at the endpoint of the study, indicated that AAJT had caused significant depletion of clotting factors (&: $P = 0.007$). F and G: According to the latest measurements, both MA (£: $P = 0.018$) and G (Ω: $P = 0.014$) showed that the stability of blood clots was negatively affected by AAJT. H and I: The trend of K ($P = 0.041$) and Angle ($P = 0.004$) over the course of the study confirmed that fibrin function in the AAJT group was compromised due to recanalization. ¥: Animals treated with AAJT showed the lowest Angle value at $T_{30min'}$ (AAJT: $68.63 \pm 7.97°$ vs. M-EHCD: $76.22 \pm 3.57°$ vs. SJT: $75.45 \pm 2.32°$, $P = 0.045$). J: A downtrend of CI value was observed in the AAJT group. §: Animals from the AAJT group had the lowest CI measurement at $T_{60min'}$ ($P = 0.048$).

hemorrhage. This NCTH model provided crucial basis for bench and bedside practices. It was also noted that this grouping method may impede an accurate evaluation of the separate impact of M-EHCD or IH on the efficacy of NCTH control.

The M-EHCD demonstrated the most rapid hemostasis ability among all groups under the current study protocol. Additionally, the significantly reduced free blood loss helped to counteract the additional bleeding resulting from multiple rounds of IH. Therefore, M-EHCD provided a secure basis for applying this novel hemostatic strategy during external NCTH control. The relative stability of SI also reflected the beneficial property of M-EHCD + IH in reducing pre-hospital hemodynamic fluctuations.

The DEP required for achieving hemostasis was significantly higher in the SJT group, whereas M-EHCD required the lowest DEP among groups. The higher IVP observed in the SJT group was partially due to the intra-peritoneal conduction of DEP [26]. In addition, the wider PE of AAJT led to a further limitation in the abdominal compliance (AC). When AC was significantly reduced, the relative increase in abdominal volume during inspiration caused by the downward movement of the diaphragm could cause a notable increase

in IVP [27]. Following a pathological rise in IVP, the blood supply within the abdominal compartment and other cavities was disrupted, leading to serious organ damages [28]. In this study, AAJT and SJT generated grade IV or II intra-abdominal hypertension [29], respectively, whereas M-EHCD comprehensively exhibited colon protection through utilizing the small PE and low DEP.

Since IRI is a fundamental hindrance to postoperative rehabilitation in NCTH patients [8], it is imperative to elucidate the impact of EHCDs removal on physiology. During the reperfusion phase, alterations in pH, Lac, and potassium levels indicated that animals in the AAJT group experienced severe acidosis. Based on the results of DD and TEG tests, AAJT exhibited a greater inclination towards secondary coagulopathy compared to M-EHCD and SJT during reperfusion. The DD measurements obtained after removing AAJT were comparable to the findings reported in existing literature [11,23]. To summarize, the above observations suggest the efficacy and safety of M-EHCD + IH in mitigating physiological and mechanical disruption, and reducing the extent of IRI.

4.2. Reasons for EHCD improvement

Several studies suggested that continuous improvement was required in the design of EHCDs to ensure safe and efficient hemostasis. The majority of participants withdrew from the study due to significant pain caused by AAJT [6,30], which also influenced their subjective assessments [6]. Moreover, damages to components such as pressure gauges presented an additional obstacle to the adoption of AAJT [10,31]. The inflation of AAJT led to extensive compression of abdominal organs. And the cranial displacement of the intestine ultimately resulted in fatal respiratory and circulatory disorders in animals [8,23].

The utilization of two separate balloons in SJT provides an opportunity to reduce excessive organ compression and to achieve bilateral hemostasis. Nevertheless, SJT follows a circular fixation design, and stable application must be parallel to the transverse axis of the torso. This implies that SJT cannot achieve simultaneous hemostasis if bleeding sites are situated in different planes. Furthermore, inflating two balloons consecutively also heightens the likelihood of delayed emergent hemostasis.

Triangular pads and metal PE are used to enhance the durability of M-EHCD. Additional data are required to substantiate the original design intent behind the M-EHCD, which may mitigate the potential risk of dislocation through the principle of triangular stability. Furthermore, the impact of adjusting the number and positioning of PEs on enhancing the capacity to meet the hemostatic requirements of individuals with diverse body shapes and bleeding regions also warrants investigation. Additionally, M-EHCD reduces the size of PE, making the localization strategy for NCTH-related vessels more clinically feasible [7].

4.3. IRI and IH: a solution to the specific disadvantage of NCTH control

Hemostatic interventions affect not only nearby organs [32], but also the barrier function of the intestinal mucosa [33], potentially leading to remote IRI caused by bacterial translocation [34]. Even with limited occlusion duration, AAJT can still cause irreversible damage to the distal limb and bladder function [11]. Compared to unilateral occlusion of the iliac artery, controlling the distal flow of the abdominal aorta might lead to considerable necrosis of the lumbar muscles and permanent motor dysfunction [35]. Similarly, REBOA can increase the likelihood of colon or lower limb IRI [36,37], which has a noteworthy correlation with the duration of blood flow obstruction [38].

EHCD and REBOA share the common objective of preventing IRI, which has become the driving force for mutual learning between the two methodologies. Ischemic pre- and post-conditioning are classified depending on the intervention timing [16,39]. However, the sudden and unpredictable nature of traumatic events limits the practicality of the former during pre-hospital care. Conversely, the latter can effectively alleviate the severity of IRI during evacuation. Additionally, IH relies exclusively on the hemostatic device, thus reducing the need for other medical resources in preventing IRI.

The combined protective effect of M-EHCD + IH observed in this study indicates that the external application of IH may yield satisfactory outcomes similar to intermittent-REBOA [15]. However, owing to variations in animal models, hemostatic techniques, frequency of IH and its individual duration, further experiments are needed to establish the balance among added blood loss, hemostatic effectiveness, and patient benefit during IH implementation.

4.4. Limitations

The current study has several limitations that should be addressed. The hemorrhagic shock model may not adequately simulate pathophysiologic processes involved in a real-life injury scenario. Furthermore, hemi-transecting the femoral artery may not fully reflect the injury mechanism of NCTH. In addition, the placement of EHCDs prior to CFA injury may not accurately reflect the total time required for EHCDs to achieve hemostasis in actual clinical settings. Due to the significant differences in morphology and anatomy between humans and swine, it is crucial to exercise caution when interpreting and extending relevant results. Besides, the mixed grouping cannot determine whether the M-EHCD or IH played a more significant role in reducing organ damage and systemic IRI.

In the follow-up studies, we will further improve the design of M-EHCD and try to establish an animal model that is more in line with the mechanism of NCTH, and then rely on a more appropriate grouping method to reveal the independent effects of next-generation M-EHCD or IH on external hemostasis. Finally, the combined effect of M-EHCD + IH on the long-term prognosis will also be determined.

5. Conclusions

In conclusion, the combination of M-EHCD and ischemic post-conditioning may represent a promising approach to enhancing the

efficacy of external NCTH control. This package solution effectively mitigated the deleterious effects typically associated with prolonged vascular occlusion, such as increased IVP and organ malperfusion. Despite an additional increase in blood loss during the performance of IH, the rapid hemostasis achieved by M-EHCD during free bleeding offset the risk of greater total blood loss. The sustained stabilization of hemodynamics within the M-EHCD group provided a physiological basis for reducing the severity of acidosis and coagulation dysfunction during reperfusion, potentially lowering the risk of the lethal triad. While further studies are needed, these findings provide a proof-of-concept evidence supporting the use of M-EHCD and IHas a viable option for improving outcomes in external NCTH management.

Ethics approval and consent to participate

This study was approved by the Laboratory Animal Welfare and Ethics Committee of the Third Military Medical University (AMUWEC20201513). All subjects were treated humanely in accordance with the principles of *The Guide for the Care and Use of Laboratory Animals*.

Consent for publication

Not applicable.

Availability of data and materials

All data included in this study are available upon request by contact with the corresponding author. We fully acknowledge the significance of data sharing as stipulated by SCI journals. However, due to the policies and confidentiality agreements followed in our laboratory, we are unfortunately unable to deposit the data in a publicly accessible repository. In addition, the establishment of this large animal model and the collection of relevant data are very difficult and costly in terms of money, time and manpower. Our team is committed to the clinical and basic research at NCTH, and we hope to continue to mine the experimental data obtained from other perspectives to make full use of the valuable data. In addition, we hope to communicate with potential data collectors to further improve the subsequent experimental design and to find out if there is a possibility of multi-center or even cross-border collaboration in NCTH research.

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CRediT authorship contribution statement

Hua-yu Zhang: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Yong Guo:** Validation, Investigation. **Dong-chu Zhao:** Validation, Investigation. **Xiao-ying Huang:** Writing – review & editing. **Yang Li:** Writing – review & editing. **Lian-yang Zhang:** Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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